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An automated flow-injection enzyme-linked immunosorbent assay for the detection of Zearalenone

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Abstract: Zearalenone (ZEN) is a mycotoxin produced by several species of Fusarium and can be found as contaminant in corn, rice and wheat. Developing an assay for detection of ZEN in these samples is crucial, since it can be used as a warning tool when analyzing agricultural products. In this work, detection of ZEN was carried out with an automated flow enzyme-linked immunosorbent assay. ZEN antibodies were covalently immobilized on Sepharose 4B beads and packed in a micro-affinity column (15 µL), which was integrated in the flow system. The assay was performed based on the ELISA format by sequential injection of ZEN sample and ZEN-HRP (tracer) to the micro-affinity column. The injected reagents were sequentially bound by the immobilized ZEN antibodies and substrate for the labeling enzyme was introduced. The absorbance of blue-green product caused from enzymatic oxidation of the substrate was measured at 405 nm. The absorbance was inversely proportional to the concentration of ZEN. With this assay we were able to detect ZEN down to 7.5 µg L⁻¹, in only 18 min. The antibody-antigen complex was dissociated using 200 mM glycine-HCl buffer (pH 2.4), to enable reusability of the column. The optimum regeneration time was 520 seconds.

Keywords: Zearalenone; Flow-ELISA; Biosensor